ENDOCRINE CONTROL OF METABOLISM IN THE LAND CRAB, GECARCINUS LATERALIS (FRÉMINVILLE). I. DIFFERENCES IN THE RESPIRATORY METABOLISM OF SINUSGLANDLESS AND EYESTALKLESS CRABS [1, 2]

DOROTHY E. BLISS

The Biological Laboratories, Harvard University and Radcliffe College, Cambridge 38, Massachusetts

Long ago eyestalk removal was observed by Zeleny (1905) to induce molting in *Uca* and by Megušar (1912) to reduce the length of the intermolt interval of *Astacus*. Many years later Abramowitz and Abramowitz (1938, 1940), Hanström (1939), Brown and Cunningham (1939), Smith (1940) and Kleinholz and Bourquin (1941) also reported compression of the intervals between successive molts in a variety of eyestalkless crustaceans. Revival of interest in the crustacean eyestalk as a molt-inhibiting center may be attributed to the discoveries in the meantime that the eyestalk contained (1) chromatophorotropically active materials (Koller, 1925, 1927; Perkins, 1928), and (2) two organs, the sinus gland and the x-organ, which gave cytological evidence of secretory activity (Hanström, 1931, 1933). Subsequent studies, primarily by Brown and his students (see Brown, 1935, 1940), yielded considerable evidence favoring the sinus gland as the principal source of these chromatophore-activating materials.

The activity of the sinus gland did not seem to be confined to chromatophore regulation. Kleinholz (1934, 1936) clearly demonstrated the response of crustacean retinal pigments to eyestalk extract, thus confirming the suggestions of Welsh (1930a, 1930b) that there is hormonal control of these pigments. Later work by Welsh (1939, 1941) pointed directly toward the sinus gland as the agent responsible. On the basis of evidence offered by Brown and Cunningham (1939), Scudamore (1942, 1947), Kyer (1942) and Bauchau (1948a), molting was added to the list of processes thought to be controlled by the sinus gland.

It was realized, however, that since the concept of hormonal regulation by the sinus glands was based to a large extent upon effects of total eyestalk removal, the critical experiment remained to be done, namely, removal of sinus glands alone. A method devised by Brown (1942) produced in sinusglandless crayfish certain responses similar to those which characterize eyestalkless animals. A removal technique, by which injury to other eyestalk tissues was minimized, was designed and employed by Kleinholz (1947). He found (1948, 1949a, 1949b) that the

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proximal pigments of the crayfish retina behaved normally after sinus gland removal. Extra-sinusglandular sources of retinal pigment hormone had not been conclusively demonstrated, since there remained the possibility, according to Kleinholz, that the pigments could also act as independent effectors. Nevertheless, here was an indication that, in one respect at least, a crustacean without its sinus glands could still be normal.

With the application by recent workers of the Kleinholz sinus gland removal technique or its various modifications, the mystery of the sinus gland has been clarified. Most of this work has been reported only in abstracts (Bliss, 1951; Frost, Saloum and Kleinholz, 1951; Havel and Kleinholz, 1951; Passano, 1951a, 1951b, 1952a; Travis, 1951b; Welsh, 1951) or in unpublished theses (Frost, 1948; Havel, 1949; Saloum, 1951; Travis, 1951a; Bliss, 1952; Passano, 1952b). The present paper summarizes physiological evidence in support of morphological observations and interpretations already presented (Bliss and Welsh, 1952). Each sinus gland of a crab should now be conceived as a storage-release center for secretory material synthesized within cells of a diffuse neurosecretory system, involving the eyestalk ganglia, the brain and perhaps the thoracic ganglionic mass. Metabolic data obtained by Kleinholz and co-workers, coupled with morphological observations by Mr. James B. Durand, Jr. of Harvard University (personal communication), suggest that a similar relationship, demonstrable in crayfish, characterizes the Macrura as well.

Materials and Methods

Specimens of *Gecarcinus lateralis* (Fréminville) were available in large numbers in Bermuda where these studies were commenced. During the major part of the work specimens were shipped periodically to Cambridge from Bermuda. Laboratory stocks were maintained at room temperature in sand-filled cement tanks, divided by wire partitions into sections suitable for 15 to 20 crabs each. During observation of a given crab, the animal was isolated in a rectangular gallon glass jar to which had been added sand and a small finger bowl or, in the case of eyestalkless crabs, a Petri dish of sea water or tap water. The crabs were observed to sit in the water and occasionally, by use of a large claw, to drink. Peanuts, lettuce and carrots were accepted by the crabs, peanuts being preferred. Mortality both during air shipment and during maintenance was very low.

Surgery

The crab was cooled in the refrigerator for about an hour before sinus gland removal. It was then supported by plasticene in a cardboard holder which in turn lay, surrounded by ice, in a Büchner funnel. Rubber sheeting was laid over the crab's back and more ice was placed on the sheeting. The Büchner funnel was so mounted in a wooden table that the funnel top was flush with the table top (see Williams, 1946). Ice melt-water ran from the funnel into a collecting pan beneath the table.

Eyestalks were ligatured with coarse surgical cotton thread. By means of a dental burr (1.5 mm. diameter), which was mounted in a handpiece attached to a foot-regulated rheostat, a hole was drilled in the mid-dorsal portion of the eye-

stalk as it lay in its orbit. After the hypodermis had been moved aside and the connective tissue sheath slit, the blue-white sinus gland was extracted by watchmakers' forceps and a small piece of plastic cover slip was sealed with paraffin into the hole. The ligature was then released. The second eyestalk was treated in the same manner.

Eyestalk removal was also preceded by cooling of the crab in the refrigerator and followed by packing of the eyestalk stub with fibrin foam (Cutter Laboratories) or Gelfoam (Upjohn Company). Mortality after either operation was negligible when the crabs were cooled sufficiently. Eyestalk removal without previous cold anesthesia was often fatal, as were operations performed when crabs had remained over an hour in the refrigerator.

Measurement of respiratory rate and respiratory quotient

For respiratory measurements use was made of a volumetric respirometer, the design of which was suggested to the writer by Dr. P. F. Scholander. All plastic and metal portions of this instrument were machined by Mr. John R. Andrews of Randolph, Massachusetts. Of similar principle is the respirometer employed by Flemister and Flemister (1951). Figure 1A shows the essential features of the instrument used by the writer.

The edges of a jar (1) of 450 cc. or 250 cc. capacity have been ground with carborundum so that, when greased and clamped, they make an air-tight seal with a ground plastic cover (2), cut from 1/2-inch Lucite. Dead air space in the animal chamber (1) has been reduced by the addition of paraffin (3). The animal chamber is clamped by a quarter-inch brass crossbar (4), which slides up and down on two threaded brass uprights mounted on either side of a Lucite platform (5). The platform is supported by a strip of brass (6). One arm of a plastic manometer (7), designed by Scholander (1949), is connected to the animal chamber. The other arm of the manometer leads to a thermobarometer (8), a bottle having a capacity of 250 cc. and containing a small amount of water. Manometer fluid, devised by Dr. Howard A. Schneiderman of Harvard University, consists of a 1:200 dilution of a concentrated liquid detergent, Aquet (Emil Greiner Company), to which is added three drops of 30% H_2SO_4 and enough acid fuchsin to give a bright red color. Acidification insures that only negligible amounts of carbon dioxide can be taken up by the manometer fluid. In the plastic cover of the animal chamber is a hole, plugged with a vaccine bottle stopper (9) and situated directly over a round, shallow plastic KOH dish (10), which is suspended by a screw from the plastic cover. To facilitate quantitative removal of the alkali, the dish is slightly funnel-shaped, has a small central depression, and is coated with a thin film of paraffin. The two arms of the manometer are open to air. When the instrument is in use, one opening is closed by a solid Lucite plug (11) and the other opening, in the arm leading to the animal chamber, is connected by a hollow Lucite taper (12) and eighth-inch plastic tubing (13) to a greased 10-milliliter hypodermic syringe (14), wired firmly to the animal chamber cover so that when the instrument is in the constant temperature bath, the syringe is under water. This syringe serves as a calibrated oxygen reservoir.

Figure 1B is a diagram of the gas analyzer used in the determination of the amount of carbon dioxide given off by a crab. To a Scholander plastic manometer

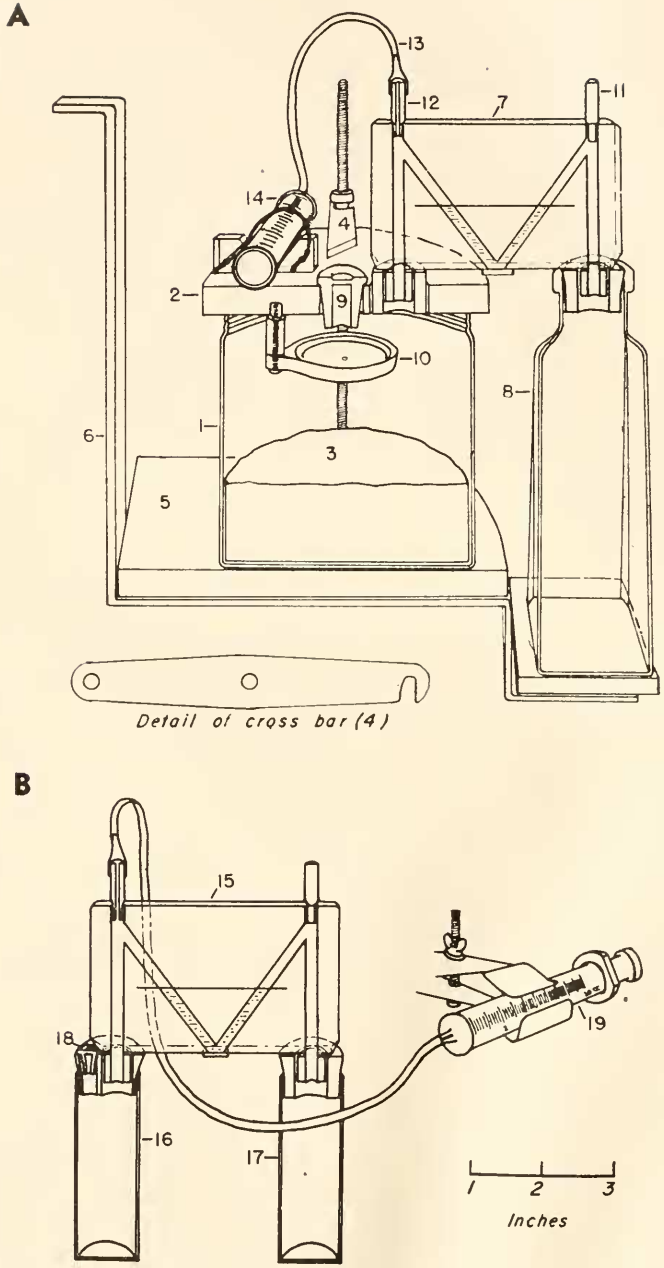

FIGURE 1. (A) Volumetric respirometer and (B) gas analyzer for volumetric determination of carbon dioxide, both drawn as if sectioned longitudinally through the midline. For explanation of figure numbers, see text.

(15) are attached two 18-milliliter glass shell vials, a dry vial (16) to act as a reaction vessel and a moistened one (17) to serve as a thermobarometer. In the rubber stopper of the reaction vessel is a small vaccine bottle stopper (18) and from the reaction vessel an arm of the manometer leads by means of plastic tubing to a 10- or 20-milliliter hypodermic syringe (19) which acts as the calibrated carbon dioxide measuring device. The manometer and shell vials are clamped in a constant temperature water bath and the carbon dioxide syringe is secured alongside the tank.

Procedure was standardized as follows: A crab was placed in the animal chamber, the instrument was assembled and clamped in the water bath, and the animal chamber was connected to the oxygen reservoir. Two milliliters of 10% KOH were injected into the shallow plastic dish through the vaccine bottle stopper just above it. After an equilibration period of 1½ to 2 hours, the KOH was removed by a hypodermic syringe, the needle point of which had been ground to a flat surface. The KOH was measured and discarded, the dish was rinsed with two milliliters of distilled water which was then quantitatively removed, and two milliliters of 10% KOH, which had been equilibrated to bath temperature (25.1° C.), were added. Immediately the first reading on the oxygen syringe was recorded. Readings to the nearest 0.05 of a milliliter were made at convenient intervals of from 10 to 45 minutes for a total time of 2½ to 4 hours. Most runs lasted about three hours and all runs were arranged to come in the afternoon, so that diurnal variations could be minimized. At the end of the run the KOH was quantitatively removed by a hypodermic syringe and injected at once into the reaction vessel of the gas analyzer. Eight-tenths of a milliliter of 30% H_2SO_4 was injected, the instrument was shaken vigorously until deflection of the manometer fluid reached a maximum (about three minutes), and the evolved carbon dioxide was measured on the attached syringe. This value was corrected for the carbon dioxide remaining in solution and for the carbonate originally present in the base. The crab was then removed from the animal chamber and weighed to the nearest tenth of a gram.

A significant portion of the carbon dioxide released from the KOH by acid remains in solution. Empirical data yielding the necessary correction

$$\left(\frac{\text{theoretical } CO_2 - \text{observed } CO_2}{\text{theoretical } CO_2}\right)$$

were obtained by the release within the gas analyzer of different volumes of carbon dioxide from standard carbonate solutions prepared so as to duplicate the carbonate-hydroxide mixtures resulting from an actual volumetric run. These standards were made by adding varying amounts (0.1–1.2 ml.) of 1.786 N K_2CO_3 to sufficient 1.786 N KOH (10% KOH) to give a total fluid volume of 2.0 ml. Gas release was accomplished by the addition of 0.8 ml. of 30% H_2SO_4 by weight (preparation: 20 ml. of concentrated H_2SO_4, 96% pure, plus 80 ml. of distilled water). Forty-eight determinations gave data in per cent which approximate the curve drawn in Figure 2. This curve was actually plotted from theoretical amounts of carbon dioxide calculated from the following formula:

$$V_t = V_o + \frac{V_o \propto V_f}{V_o + V_s},$$

where V_t represents total CO_2, V_o is observed CO_2, V_f is the volume of fluid (2.8 ml.), V_s is the gas capacity of the shell vial (14.2), and α represents the solubility coefficient for CO_2 at 25 degrees C. (0.570). This solubility coefficient was determined from available data (Seidell, 1940) for potassium acid sulfate and sulfuric acid of the same ionic strength as the solution resulting from the reaction between the acid and KOH–K_2CO_3. The solution was calculated to be approximately 3 N for both H_2SO_4 and $KHSO_4$. Although this equation was formulated independently, it is identical, when simplified, with that developed by Scholander, Claff, Andrews and Wallach (1952).

These theoretical and empirical results indicate that, under the stated experimental conditions, the correction which must be applied to an observed volume of carbon dioxide is almost 9% at a volume of 2.5 ml. but decreases to 4% at 24.0 ml. (Fig. 2). It should be emphasized that the position of the curve and therefore the per cent correction vary with temperature, concentration and amount of KOH–K_2CO_3, concentration and amount of H_2SO_4, and gas capacity of the reaction chamber. The carbon dioxide values from which the respiratory quotients

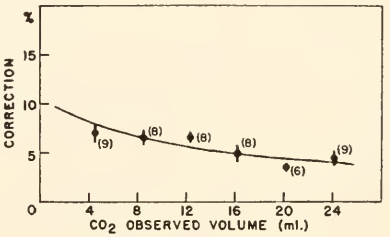

FIGURE 2. Theoretical curve and empirical data showing the correction in per cent which, due to the solubility of carbon dioxide in salt solutions, must be applied to observed volumes when carbon dioxide is evolved under the experimental conditions described in the text. Arithmetic means are indicated by closed circles and standard errors by vertical lines through these circles. Number of determinations indicated in parentheses.

presented in this paper were calculated have been corrected for the amount of carbon dioxide remaining in solution. Consequently, respiratory quotients are somewhat higher than those reported previously (Bliss, 1951, 1952) from the same respiratory data, since at the time of writing of those papers the error had not been recognized and determined.

Blank volumetric runs, performed under conditions identical with those of an experimental respiratory run but without the crab in the animal chamber, revealed that about 1½ hours were required for the respirometer to become equilibrated to the temperature of the water bath. After equilibration had been completed, blank runs of 8 hours' duration produced no measurable deflections of the manometer fluid. As indicated earlier, experimental procedure was standardized to include 1½ to 2 hours of equilibration.

This prolonged period of temperature equilibration served to insure chemical equilibration of the crab's body fluids with the carbon dioxide in transit between the animal and the KOH. A modification of the method described by Scholander, Claff, Andrews and Wallach (1952) was employed in determination of the amount

of carbon dioxide in transit at any given carbon dioxide output rate. Carbon dioxide was released by 0.8 ml. of 30% H_2SO_4 from 2 ml. of KOH–K_2CO_3 in a Stender dish situated within the animal chamber of the respirometer. The volume of evolved gas, about 9.5 ml., was noted on the ten-milliliter greased syringe used for injection of the acid, the base of the syringe needle having, prior to the injection, been sealed into Tygon tubing and pushed firmly into the manometer port leading to the animal chamber. Two milliliters of 10% KOH were injected into the KOH dish. The carbon dioxide uptake by this KOH was followed on the syringe and plotted as a function of time. Tangents drawn to the carbon dioxide uptake curve, as described by Scholander, Claff, Andrews and Wallach (1952), indicated the amount of carbon dioxide in transit. This gas in transit, reaching concentrations within the animal chamber as high as 0.2% of the total gas volume during the respiration of a normal crab and up to 0.7% for a molting eyestalkless crab, can lower the total observed carbon dioxide and secondarily the respiratory quotient (1) by being untrapped in the alkali, and (2) by causing some carbon dioxide to be retained within the buffered body fluids of the crab. For these reasons, in the method described here, the KOH of the equilibration period does not contain the total amount of carbon dioxide produced by the crab during this period. However, this alkali is discarded. The KOH of the experimental run is added after the carbon dioxide of the crab's body fluids, the carbon dioxide in transit, and the carbon dioxide being trapped by the alkali have reached a steady state. Therefore all carbon dioxide produced by a crab during an experimental run is trapped in the KOH added at the beginning of the run. The recorded amount of carbon dioxide evolved from this KOH, when corrected for the gas remaining in solution, is a true measure of total carbon dioxide production during the experiment.

In order that the carbon dioxide buffer capacity of a crab might be estimated, the total oxygen consumption of two normal crabs was recorded first in the presence of KOH and immediately thereafter in its absence. The magnitude of the difference between these amounts, less than expected on the basis of an average respiratory quotient of 0.7 for these crabs as determined by the gas analysis method, was an indication of the power of a crab's body fluids to hold carbon dioxide in chemical combination. Clearly essential to the procurement of valid respiratory data is one of the following: (1) a low tension of carbon dioxide in transit; (2) an extended period of experimental observation and measurement; or (3), as in the method described here, a renewal of the carbon dioxide-absorbing KOH after a prolonged period of equilibration.

During experimental runs either with or without KOH, no sudden deflections of the manometer fluid, indicative of "bursts" of carbon dioxide, were observed. Such "bursts" have been described for insects by Punt (1950) and by H. A. Schneiderman and C. M. Williams (personal communication). The carbon dioxide output of a land crab at 25 degrees C. is steady and continuous. Carbon dioxide output by a land crab, at least at this temperature, can be taken as a true measure of its carbon dioxide production. If the gas is properly trapped by alkali and subsequently released from the alkali and measured, and if the measurement is corrected for solubility error, a valid respiratory quotient can be calculated.

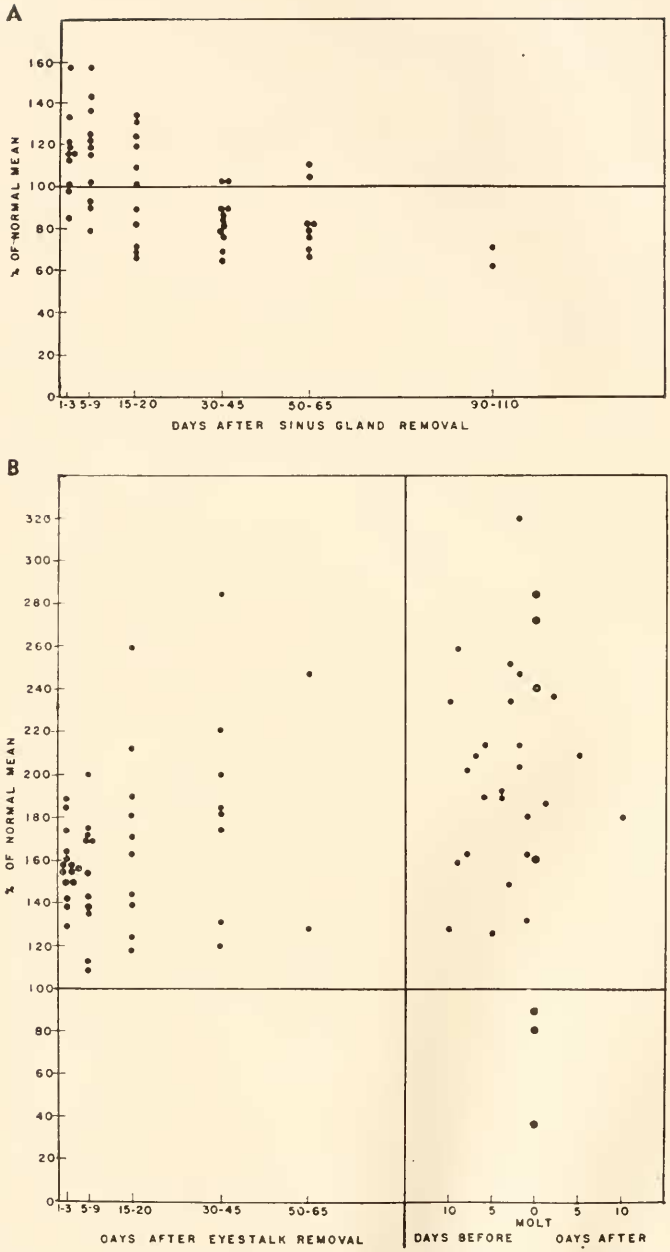

FIGURE 3. Rates of oxygen consumption of *Gecarcinus lateralis* after (A) bilateral removal of sinus glands and (B) bilateral eyestalk removal. Each value is plotted as per cent of the normal mean for that animal.

Observations

Contrasts in Metabolism Between Sinusglandless and Eyestalkless Crabs

1. *Respiratory rate*

Rate of oxygen consumption was determined as $mm.^3$ per gram live weight per hour under standard conditions. Since it has been shown for the fiddler crab by Edwards (1950) that the absolute rate varies with sex and size, runs were limited to male animals, and all graphs intended to compare rates of many different animals have been expressed in per cent of each animal's normal average, as determined in two to four control runs.

Figures 3A and 3B indicate the striking differences between the rates of oxygen consumption of crabs from which only sinus glands have been removed and the respiratory rates of animals from which entire eyestalks have been taken. Figure 3A shows that after bilateral sinus gland removal, the rates of oxygen consumption tend in some animals to be high for the first few days but to be normal again within ten days to two weeks. The respiratory rates continue to drop, so that by the end of the first month they are below normal.

Bilateral eyestalk removal, on the other hand, causes the rates of oxygen consumption of all crabs to rise abruptly so that by the next day they are from 130% to 190% of normal. This rise may be followed by a temporary minor drop and then there is a gradual increase up to molt. On the day of molting, the respiratory rates have been variously recorded as 284% down to 36% of normal, but it is significant that the rates of those crabs which survived their molt were 284%, 273% and 240% of normal, whereas rates falling in the lower percentage range were recorded on animals which died during molt. It may be concluded that the respiratory rates are maximal at the time of molt. These observations on eyestalkless land crabs agree with those of Scudamore (1947) on crayfish and of Edwards (1950) on fiddler crabs. Scudamore, using the technique of Brown (1942), found that removal of sinus glands from three crayfish caused an increase in rates of oxygen consumption from a quarter to a fifth as great as the increase following eyestalk removal. Since his observations were limited to the first four days after surgery, he was probably observing in *Cambarus* a rise in respiratory rate comparable to that which the present writer has found may or may not occur in *Gecarcinus* following sinus gland removal and which, in any case, has disappeared within the ten days to two weeks following the operation.

2. *Respiratory quotient*

Each respiratory quotient was calculated as the total amount of carbon dioxide produced divided by the total amount of oxygen consumed during an experimental run.

The respiratory quotients of the entire series of normal animals tested from the beginning of November, 1950, until the end of June, 1951, are plotted in Figure 4. Each circle represents an individual determination and two to four separate circles represent the respiratory quotients for one animal. Therefore the one hundred and forty-odd circles represent the respiratory quotients of about forty animals. There is considerable variation in the normal respiratory quotient, which may range as high as 0.97 or as low as 0.61. The standard deviation of

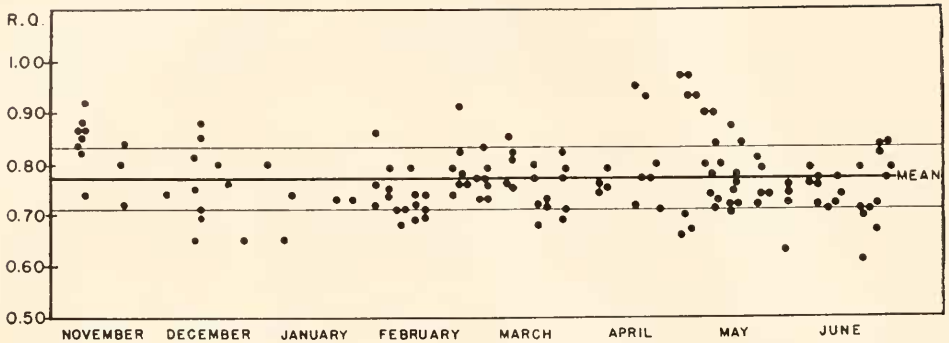

FIGURE 4. Respiratory quotients recorded from normal *Gecarcinus lateralis* from November, 1950, through June, 1951. The heavy line represents the mean respiratory quotient and the two narrow lines on either side of the mean indicate the range of plus and minus one standard deviation.

the mean respiratory quotients of 37 crabs is ± 0.06. The mean value is 0.77 ± 0.01.

In Figure 5A are shown the respiratory quotients for crabs from which both sinus glands have been removed. Each circle on the graph represents an individual determination but in this case the values are grouped, so that all determinations made between the first and the third day after sinus gland removal are in one column, those made between the fifth and ninth days in another, and so on. These values have an essentially normal distribution although there is a tendency, probably not of statistical significance (Table I), for a drop in respiratory quotient

TABLE I

The respiratory quotients of Gecarcinus lateralis

	Number in sample	Arithmetic mean and standard error	Probability (P)*
Normal Crabs	37	0.77 ± 0.01	
Eyestalkless Crabs			
1–3 days after operation	7	0.63 ± 0.02	<0.001
5–9 days after operation	6	0.69 ± 0.01	0.001
7–20 days pre-molt	16	0.68 ± 0.01	<0.001
Molting	7	1.40 ± 0.14	<0.001
Sinusglandless Crabs			
1–3 days after operation	10	0.72 ± 0.02	0.02
5–9 days after operation	11	0.74 ± 0.02	0.13
15–20 days after operation	11	0.79 ± 0.02	0.43
30–45 days after operation	11	0.77 ± 0.02	0.93
50–65 days after operation	8	0.75 ± 0.03	0.28

* P values are from table of t as given in Fisher and Yates (1948). A difference in mean respiratory quotient between normal and operated crabs is considered highly significant if P is less than 0.01.

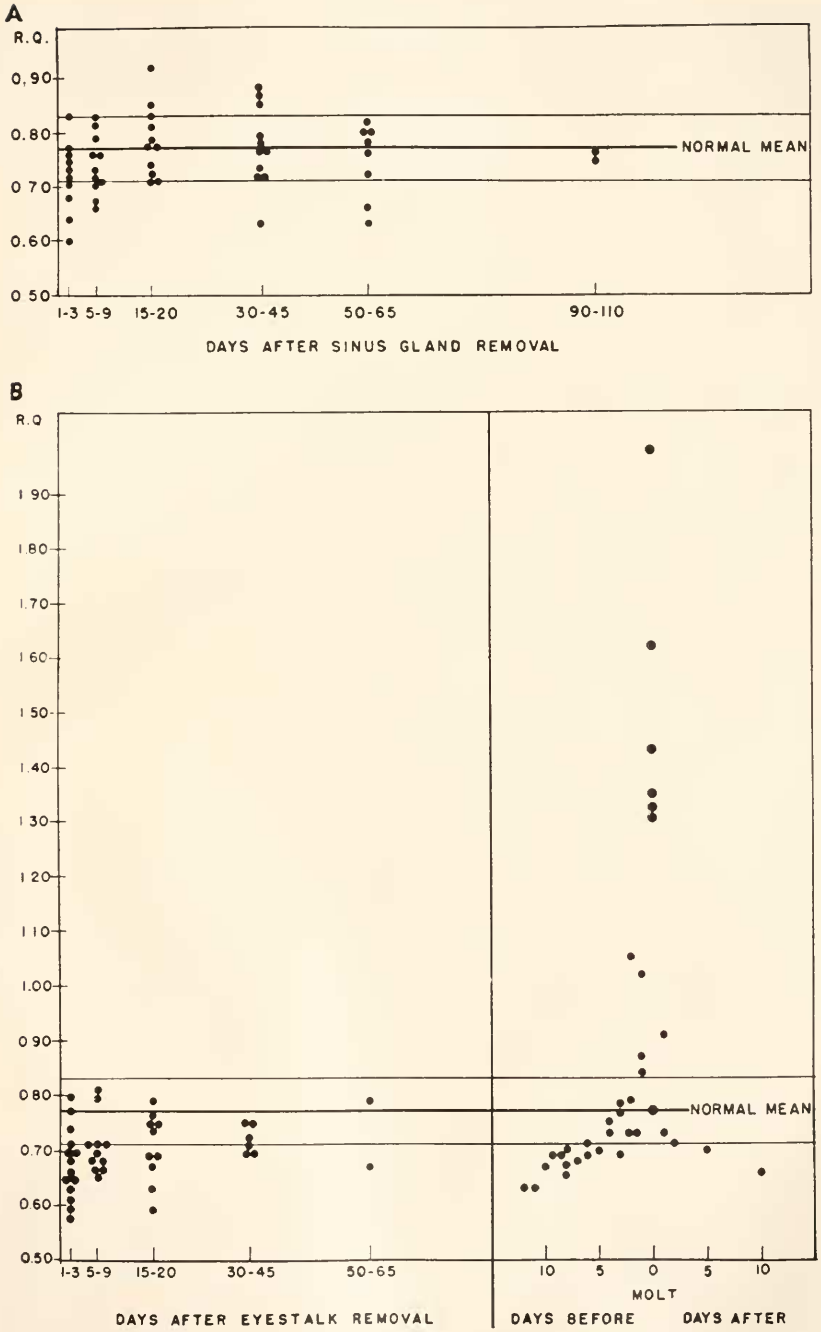

FIGURE 5. Respiratory quotients of *Gecarcinus lateralis* after (A) bilateral removal of sinus glands and (B) bilateral eyestalk removal. The normal mean and standard deviation are indicated as in Figure 4.

during the first few days after the operation, then a recovery to or above normal, and finally a leveling at approximately the normal mean value.

Removal of both eyestalks causes a radical change in respiratory quotient (Fig. 5B). Three days after operations performed in the spring months, the mean respiratory quotient has dropped to 0.63 ± 0.02 (Table I). By the fifth day the mean has risen somewhat to 0.69 ± 0.01, remaining near this value until around the tenth day before molt. A gradual rise, which starts at this time, culminates in a precipitous increase on the day of molt, when values as high as 1.97 have been recorded. Subsequently the mean respiratory quotient falls to the pre-molt level.

The rapidity and magnitude of response to eyestalk removal depends upon the time of year at which the operation is performed. Operations on four crabs during January and February produced no clear effect on respiratory quotients. Three weeks after the operations, the respiratory quotients of these crabs dropped to values which, although subnormal, were generally higher than those recorded from crabs made eyestalkless during the spring. The January-February data are responsible for the circles in Figure 5B (left side) which lie close to the line representing the normal mean. Similarly rates of oxygen consumption from these crabs are indicated by the circles in Figure 3B nearest to the 100% line. Since respiratory quotients recorded in January and February were from crabs which were not immediately responsive to eyestalk removal, these data have been omitted from calculations for respiratory quotients typical of crabs 1–3 and 5–9 days after eyestalk removal (Table I). It is interesting to note, however, that when data from non-responsive eyestalkless crabs of the winter months are included in the calculations, the mean respiratory quotients of 0.67 ± 0.02 for 1–3 days and 0.71 ± 0.01 for 5–9 days are still significantly below the normal mean of 0.77 ± 0.01, with P values of less than 0.001 and 0.01, respectively.

Intervals between eyestalk removal and molt become shorter with the advance of the seasons. In January and February forty to fifty days intervene between operation and molt, whereas in June only twenty to thirty days elapse. One may postulate that in the spring, eyestalk removal throws a crab at once into a full-fledged growth and molt metabolism, with an accompanying drop in respiratory quotient and rise in respiratory rate. During the winter months this shift in metabolism is partially blocked, so that the growth processes which are triggered by eyestalk removal start more slowly, proceed at reduced rates, and require longer periods of time for their completion.

3. *Water uptake and body weight*

Increases in water uptake and weight were first reported by Scudamore (1947) for eyestalkless crayfish and for crayfish from which sinus glands had been removed. Figure 6A shows that removal of both sinus glands from *Gecarcinus lateralis* did not cause the weight of the crabs to deviate significantly from normal. Increase in weight followed eyestalk removal, the greater portion of the increase commencing about 10 days before molt, with maximum values on the day of molt (Fig. 6B).

Responsible for the pre-molt weight increase in eyestalkless crabs was their

water uptake, becoming so marked as molt approached that the membrane linking abdomen to thorax bulged as if about to burst. The weights recorded on sinus-glandless crabs remained approximately unchanged because no increase in water uptake occurred. Intersegmental membranes were normal in appearance.

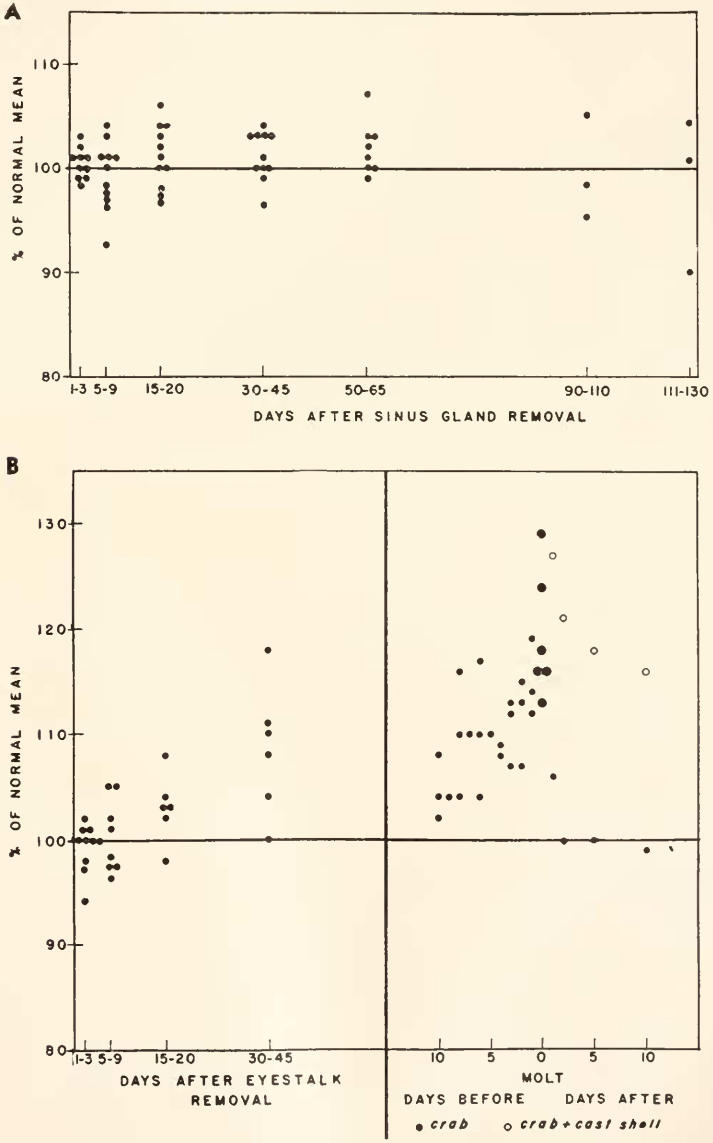

FIGURE 6. Wet weights of *Gecarcinus lateralis* after (A) bilateral removal of sinus glands and (B) bilateral eyestalk removal. Each value is plotted as per cent of the normal mean for that animal.

4. *Molt and gastrolith formation*

From the observations summarized in the preceding paragraphs it is apparent that, along with the relatively normal respiratory rate, respiratory quotient and water uptake which characterize sinusglandless crabs, there is no induction of molt. Eyestalk removal causes molt, with its preliminary alterations in respiratory metabolism and water balance, to occur. During the period of experimentation and observation (November, 1950, through June, 1951) only one normal crab molted.

Coincident with other preparations for molt, eyestalkless crabs remove calcium from the exoskeleton and deposit it as gastroliths under the chitinous lining of the stomach. Although from about the tenth day after eyestalk removal four pearly-white gastroliths form upon the calcium carbonate framework of the stomach, they do not appear after sinus gland removal. Scudamore (1947, p. 192) reported that in crayfish "bilateral sinus-gland extirpation resulted in gastrolith formation in all cases, although at a slightly slower rate than after bilateral eyestalk ablation." His data, however, reveal that 17 days after surgery the gastroliths of sinusglandless crayfish weighed only 23% as much as did those of eyestalkless animals.

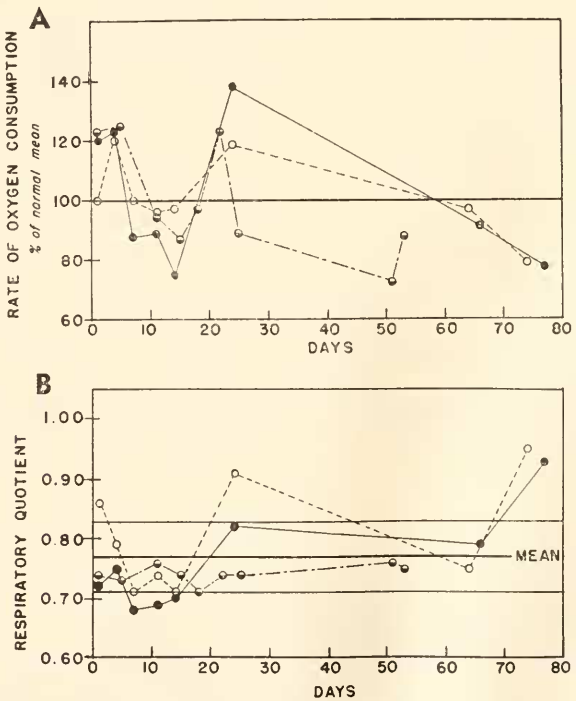

FIGURE 7. (A) Rates of oxygen consumption and (B) respiratory quotients of three normal crabs recorded over periods up to 77 days. The respiratory rates are indicated as per cent of each animal's mean. The normal mean respiratory quotient and standard deviation are indicated as in Figure 4.

The Respiratory Metabolism of Individual Crabs

The general pattern of response that is visible in group data presented in the previous section is apparent when the respiratory metabolism of individual crabs is followed for long periods.

1. *Normal respiratory metabolism*

In Figure 7A are plotted the rates of oxygen consumption of three crabs over a period of 77 days. Figure 7B shows the corresponding respiratory quotients during the same respiratory runs. Visible in both graphs is considerable fluctuation, indicating that a normal crab varies both its rate and type of metabolism. From a mean respiratory quotient of 0.77, it can be tentatively assumed that the foods principally oxidized are proteins and fats. Oil globules are visible in great quantities in the hepatopancreas. Fluctuations of respiratory quotient suggest, however, that conversion of carbohydrate to fat, producing higher respiratory quotients, and conversion of fat to carbohydrate, yielding lower values, may be involved in normal maintenance metabolism. The lower, steadier respiratory quotients recorded after eyestalk removal suggest that the fluctuating metabolism of the normal crab has been replaced by a relatively invariable metabolism, in

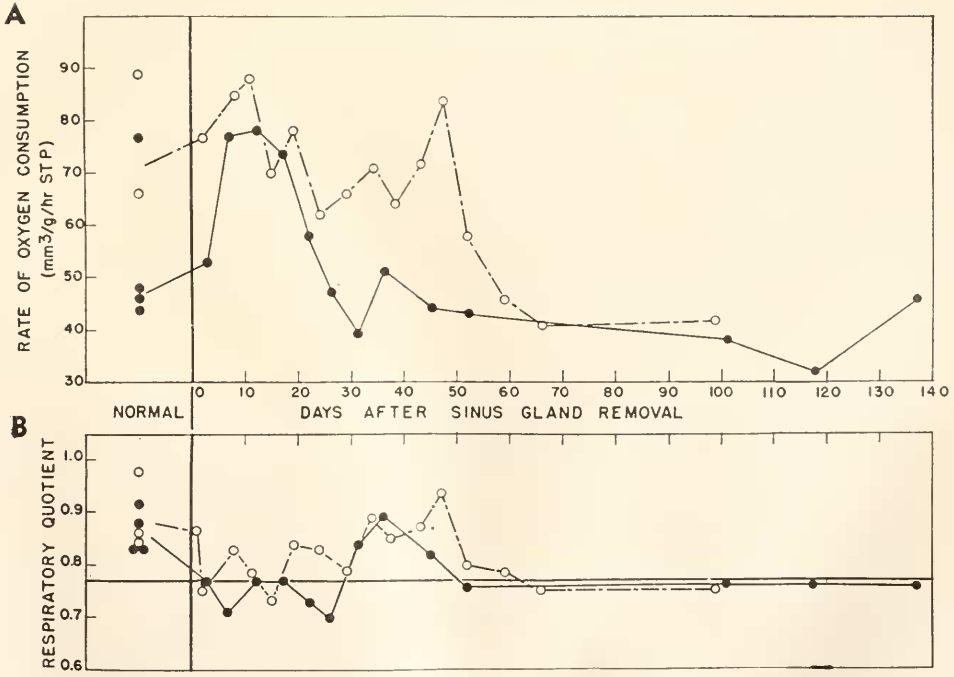

FIGURE 8. (A) Rates of oxygen consumption and (B) respiratory quotients of two crabs when normal and after bilateral removal of sinus glands. Open circles: crab 2; closed circles: crab 4. Solid horizontal line in (B): normal mean respiratory quotient.

which oxidation of fats and conversion of fats into organic acids, carbohydrates and other raw materials for the synthesis essential to growth play important roles.

2. *Respiratory metabolism after bilateral sinus gland removal*

One to two months after bilateral sinus gland removal there occurred stabilization of the respiratory quotient (Fig. 8B) close to the normal mean of 0.77 and of the respiratory rate (Fig. 8A) at normal or sub-normal levels. In six out of eight cases, of which one (crab 4) is shown in Figure 8A and another (crab 26) is illustrated in Figure 9C, the respiratory rate ascended immediately after the operation and then declined almost without fluctuations to reach, within the first month, a steady normal or sub-normal level. In the remaining two cases, of which one is illustrated in Figure 8A (crab 2), the respiratory rate subsequent to sinus gland removal varied near this crab's normal mean before stabilizing at a steady low level.

In four out of eight sinusglandless crabs, including crabs 2 and 4 of Figure 8B, the respiratory quotient fluctuated around the normal mean before stabilizing there, but in the remaining four individuals, of which one (crab 26) is shown in Figure 9D, the respiratory quotient fell sharply immediately after sinus gland removal and within three weeks had climbed smoothly to normal, where it stabilized.

In a previous paper (Bliss and Welsh, 1952) it was reported that a sinus gland starts to regenerate in an atypical position on the medulla terminalis of each eyestalk shortly after bilateral sinus gland removal. It was demonstrated that normal and regenerated "glands" are masses of swollen nerve endings containing secretory material which has been produced in neurosecretory cells of the central nervous system and transported along their axons to these reservoirs. By the time the respiratory metabolism has stabilized, the crab has substitute sinus glands. Yet regenerated glands of one or two months cannot be functioning like the original structures, since the respiratory metabolism is not fluctuating as it does in a normal animal. The long-term respiratory picture suggests that sooner or later a crab from which the original sinus glands have been removed is without the ability to vary the amount and the type of its metabolism.

3. *Respiratory metabolism after removal of one eyestalk*

Removal of one eyestalk may produce a slight rise in rate of oxygen consumption or, as in crab 21 (Fig. 9A), no change. A temporary drop in respiratory quotient may occur (Fig. 9B). Subsequent removal of the other eyestalk from the same crab causes a permanent pre-molt rise in respiratory rate and fall in respiratory quotient, as in other eyestalkless crabs (Figs. 3B, 5B).

Bauchau (1948b) obtained comparable results in *Eriocheir sinensis*, observing a 25% increase in respiratory rate five days after unilateral eyestalk removal and a sharp rise after removal of the second eyestalk. Edwards (1950) reported a respiratory rate 36% above normal when *Uca* from which one eyestalk had been removed were tested 14 to 39 days after the operation. The rate rose to 162% of normal when the second eyestalk was ablated.

The small rise observed by Bauchau after unilateral eyestalk removal was at-

tributed by him to insufficiency of secretion from the remaining sinus gland. If this interpretation is correct, Edwards' results indicate that compensation for the shortage of hormone by release of gradually increasing amounts from the intact gland does not occur. The slight intensification of respiratory metabolism correlates with the slight acceleration of molt frequency observed by Brown and Cunningham (1939) in crayfish possessing only one eyestalk.

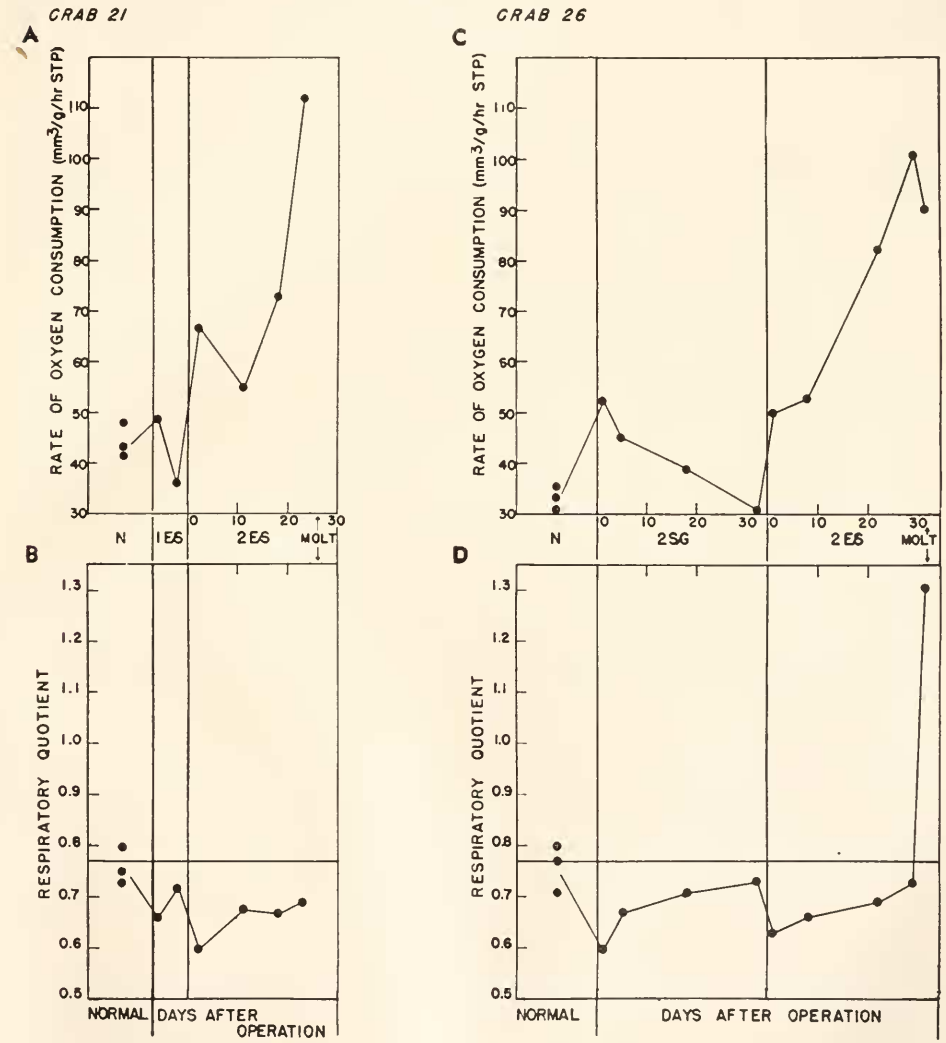

FIGURE 9. (A) Rates of oxygen consumption and (B) respiratory quotients of crab 21 when normal (N), after removal of one eyestalk (1ES) and after removal of the second eyestalk (2ES). The respiratory rates (C) and respiratory quotients (D) of crab 26 are illustrated when the crab is normal (N), without its sinus glands (2SG), and later without its eyestalks (2ES). Solid horizontal line in (B) and (D) : normal mean respiratory quotient.

4. *Respiratory metabolism when bilateral sinus gland removal is followed by bilateral eyestalk removal*

The usual responses to eyestalk removal occur just as if there had been no previous surgical treatment, when bilateral eyestalk removal follows some time after bilateral sinus gland removal (Figs. 9C, 9D). These responses, recorded in three crabs, indicate that (1) the ability to respond to eyestalk removal is not altered by previous sinus gland removal, and (2) the molt-inhibitory hormone is permanently withdrawn or effectively reduced in concentration not by sinus gland removal but by eyestalk removal.

Discussion

Marked differences between the effects on respiration of sinus gland removal and those of eyestalk removal, coupled with contrasts in water uptake, gastrolith formation and induction of molt, have led the writer inevitably to the conclusion that the removal of sinus glands in *Gecarcinus lateralis* is not equivalent to bilateral eyestalk removal. Observations by Passano (1951b, 1952b) on the incidence of molting in crabs after eyestalk removal and after sinus gland removal (technique modified from Kleinholz, 1947) are in complete harmony with the results reported in the present paper. Furthermore, Passano and the writer separately have found that the sinus gland of crabs is connected by a large nerve tract with the x-organ [3] (Passano, 1951a, 1951b, 1952b; Bliss, 1951, 1952). Passano (1951b, 1952b), in an extensive series of experiments has shown that x-organ removal, like eyestalk removal, can induce molt and that implants of x-organs into eyestalkless crabs can delay molting. Passano has demonstrated for the first time a function, molt inhibition, for the x-organ. Results of two experiments by the present writer are in agreement with this work of Passano. Bilateral removal of x-organ and x-organ nerve, in one case with and in the other case without simultaneous removal of sinus glands, brought the same respiratory and molting responses in *Gecarcinus lateralis* as did bilateral eyestalk removal. On the basis of physiological and morphological evidence it was suggested independently by Bliss and Passano that a hormone [4] is synthesized in the x-organ of crabs and transported by way of a nerve to the sinus gland. Subsequent studies by Bliss and Welsh (1952) have demonstrated morphologically that many groups of neurosecretory cells situated in ganglia throughout the central nervous system of crabs participate in the synthesis of secretory material and in its transport along their axons to the two storage-release centers, the sinus glands. These more recent observations can explain the effectiveness of certain eyestalk tissues other than x-organs in delaying molt when, as reported by Passano (1951b, 1952b), they are implanted into eyestalkless crabs.

The conclusions presented in this paper that processes triggered by bilateral eyestalk removal are not evoked by bilateral sinus gland removal contrast with conclusions published by Scudamore (1947). Scudamore wrote (p. 205), "Bi-

[3] Probably the same discovery as was made in *Sesarma* by Enami (1951) who described these neurosecretory cells as "beta cells."

[4] Other than to suggest that the molt-inhibiting and respiration-regulating principles may be identical, the present studies have led to no conclusions concerning the number, identity and interrelationships of hormones being released by the sinus gland.

lateral eyestalk or sinus-gland excision resulted in gastrolith formation, removal of soluble salts from the exoskeleton, increase in water content, increase in oxygen consumption, and molting. . . ." *Cambarus,* used by Scudamore, responded to sinus gland removal (at 20% to 40% of the eyestalkless crayfish response with, however, only one molt) whereas, with the exception of a temporary change in mean respiratory rate and quotient, *Gecarcinus,* used by the present writer, did not respond in a molt-preparatory manner. In the light of recent physiological and morphological observations on crayfish, summarized in the next paragraph, it is probable that these contrasting results may be explained in terms of differing techniques of sinus gland removal. Proximity of the sinus gland to many neurosecretory centers within the eyestalk (see Bliss and Welsh, 1952, Figs. 1–3) requires that destruction of eyestalk tissue either during or following an operation be minimized. The method of sinus gland removal described in the present paper has the advantage, in common with the technique devised by Kleinholz (1947), of facilitating removal of a sinus gland with little derangement of other eyestalk tissues.

There are now indications that in crayfish the sinus glands can be removed without permanent interference with metabolism. Normal blood calcium levels were maintained and no incidence of molting occurred in crayfish from which sinus glands had been removed (Havel, 1949; Havel and Kleinholz, 1951). Sinus glandless crayfish were found to have a rate of oxygen consumption which was only slightly higher than that recorded after mock sinus gland operation and considerably below that of eyestalkless animals (Frost, 1948; Saloum, 1951; Frost, Saloum and Kleinholz, 1951). Mr. James B. Durand, Jr. (personal communication) has observed that nerve fibers from the x-organ and brain of crayfish carry stainable secretory material to their swollen endings, the sinus glands. In Macrura as in Brachyura there appears to be a neurosecretory system in which the sinus glands play the role of storage-release centers. The concept of a neurosecretory system, developed originally for vertebrates and insects (Scharrer and Scharrer, 1944; Bargmann and Scharrer, 1951; Scharrer, 1951; Scharrer, 1952a, 1952b) has now been extended to include the decapod Crustacea (Bliss and Welsh, 1952).

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Summary

1. A volumetric macrorespirometer and gas analyzer are described. Procedures and precautions for their use are discussed.

2. Determinations of respiratory rates and respiratory quotients before and after surgical removal of both sinus glands from the eyestalks of the land crab, *Gecarcinus lateralis,* indicated that, except in one respect, the crabs were fundamentally unaffected by the loss of organs which had been thought essential for maintenance of normal metabolism.

3. The exception lay in an operated crab's eventual loss of its normal ability to vary the type and rate of metabolism. Indications of this loss were a constant respiratory quotient at the normal mean value and a low and relatively invariable respiratory rate. Fluctuations in rate of oxygen consumption and level of respiratory quotient were recorded from normal crabs.

4. Eyestalkless crabs showed a sudden and pronounced alteration from normal respiratory rates and respiratory quotients, a change indicative of a pre-molt metabolism and culminating in a second, even more marked metabolic shift at the time of molt.

5. It is clear that removal of eyestalks does, but removal of sinus glands does not, deprive these crabs of the molt-inhibiting and respiration-regulating hormone. Previous bilateral sinus gland removal does not alter the capacity of a crab to respond to subsequent bilateral eyestalk removal in the manner described above. The same is true when unilateral eyestalk removal is followed by removal of the other eyestalk.

6. These metabolic data supplement and confirm morphological observations reported in an earlier paper (Bliss and Welsh, 1952). One may conceive of a brachyuran neurosecretory system composed of neurosecretory cell bodies as synthesizing elements, axons as transporting elements, and the sinus glands as storing and releasing organs. This is an extension of a concept developed originally by Scharrer and Scharrer (1944) for vertebrates and insects.

LITERATURE CITED

Abramowitz, A. A., and R. K. Abramowitz, 1938. On the specificity and related properties of the crustacean chromatophorotropic hormone. *Biol. Bull.*, **74**: 278–296.

Abramowitz, R. K., and A. A. Abramowitz, 1940. Moulting, growth, and survival after eyestalk removal in *Uca pugilator*. *Biol. Bull.*, **78**: 179–188.

Bargmann, W., and E. Scharrer, 1951. The site of origin of the hormones of the posterior pituitary. *Amer. Sci.*, **39**: 255–259.

Bauchau, A. G., 1948a. Phénomènes de croissance et glande sinusaire chez *Eriocheir sinensis*. H. M. Edw. *Ann. Soc. Roy. de Zool. de Belg.*, **79**: 125–131.

Bauchau, A. G., 1948b. Intensité du métabolisme et glande sinusaire chez *Eriocheir sinensis*. H. M. Edw. *Ann Soc. Roy. de Zool. de Belg.*, **79**: 73–86.

Bliss, Dorothy E., 1951. Metabolic effects of sinus gland or eyestalk removal in the land crab, *Gecarcinus lateralis*. *Anat. Rec.*, **111**: 502.

Bliss, Dorothy E., 1952. Endocrine control of metabolism in the decapod crustacean, *Gecarcinus lateralis*. Thesis for Ph.D. degree, Radcliffe College.

Bliss, Dorothy E., and J. H. Welsh, 1952. The neurosecretory system of brachyuran Crustacea. *Biol. Bull.*, **103**: 157–169.

Brown, F. A., Jr., 1935. Control of pigment migration within the chromatophores of *Palaemonetes vulgaris*. *J. Exp. Zool.*, **71**: 1–15.

Brown, F. A., Jr., 1940. The crustacean sinus gland and chromatophore activation. *Physiol. Zool.*, **13**: 343–355.

Brown, F. A., Jr., 1942. Sinus gland extirpation in the crayfish without eyestalk removal. *Proc. Soc. Exp. Biol. Med.*, **50**: 295–297.

Brown, F. A., Jr., and Ona Cunningham, 1939. Influence of the sinus gland of crustaceans on normal viability and ecdysis. *Biol. Bull.*, **77**: 104–114.

Edwards, G. A., 1950. The influence of eyestalk removal on the metabolism of the fiddler crab. *Physiol. Comp. Oecol.*, **2**: 34–50.

Enami, M., 1951. The sources and activities of two chromatophorotropic hormones in crabs of the genus *Sesarma*. II. Histology of incretory elements. *Biol. Bull.*, **101**: 241–258.

Fisher, R. A., and F. Yates, 1948. Statistical tables for biological, agricultural and medical research. 3rd ed. Edinburgh.

Flemister, L. J., and Sarah C. Flemister, 1951. Chloride ion regulation and oxygen consumption in the crab *Ocypode albicans* (Bosq). *Biol. Bull.*, **101**: 259–273.

Frost, R. C., 1948. The role of the sinus gland in relation to oxygen consumption in the crayfish. Thesis for A.B. degree, Reed College.

Frost, R., R. Saloum and L. H. Kleinholz, 1951. Effect of sinus gland and of eyestalk removal on rate of oxygen consumption in *Astacus*. *Anat. Rec.*, **111**: 572.

Hanström, B., 1931. Neue Untersuchungen über Sinnesorgane und Nervensystem der Crustaceen. I. *Morphol. u. Ökol. d. Tiere*, **23**: 80–236.

Hanström, B., 1933. Neue Untersuchungen über Sinnesorgane und Nervensystem der Crustaceen. II. *Zool. Jahrb. Abt. Anat. Ont. Tiere*, **56**: 387–520.

Hanström, B., 1939. Hormones in invertebrates. Oxford Univ. Press.

Havel, Virginia J., 1949. Some aspects of calcium metabolism in relation to molting in Crustacea. Thesis for A.M. degree, Reed College.

Havel, Virginia J., and L. H. Kleinholz, 1951. Effect of seasonal variation, sinus gland removal, and eyestalk removal on concentration of blood calcium in *Astacus*. *Anat. Rec.*, **111**: 571.

Kleinholz, L. H., 1934. Eye-stalk hormone and the movement of distal retinal pigment in *Palaemonetes*. *Proc. Nat. Acad. Sci.*, **20**: 659–661.

Kleinholz, L. H., 1936. Crustacean eye-stalk hormone and retinal pigment migration. *Biol. Bull.*, **70**: 159–184.

Kleinholz, L. H., 1947. A method for removal of the sinus gland from the eyestalks of crustaceans. *Biol. Bull.*, **93**: 52–55.

Kleinholz, L. H., 1948. Factors controlling the migration of the proximal pigment of the crustacean retina. *Anat. Rec.*, **101**: 665.

Kleinholz, L. H., 1949a. Responses of the proximal retinal pigment of the isolated crustacean eyestalk to light and to darkness. *Proc. Nat. Acad. Sci.*, **35**: 215–218.

Kleinholz, L. H., 1949b. Migrations of the retinal pigments and their regulation by the sinus gland. *Bull. Biol. de France et de Belg., Suppl.*, **33**: 127–138.

Kleinholz, L. H., and Emma Bourquin, 1941. Effects of eyestalk removal on decapod crustaceans. *Proc. Nat. Acad. Sci.*, **27**: 145–149.

Koller, G., 1925. Farbwechsel bei *Crangon vulgaris*. *Verh. deutsch. zool. Gesells.*, **30**: 128–132.

Koller, G., 1927. Über Chromatophorensystem, Farbensinn und Farbwechsel bei *Crangon vulgaris*. *Zeitschr. vergl. Physiol.*, **5**: 191–246.

Kyer, D. L., 1942. The influence of the sinus glands on gastrolith formation in the crayfish. *Biol. Bull.*, **82**: 68–78.

Megušar, F., 1912. Experimente über den Farbwechsel der Crustacean. *Arch. f. Entw.*, **33**: 462–665.

Passano, L. M., 1951a. The X organ-sinus gland neurosecretory system in crabs. *Anat. Rec.*, **111**: 502.

Passano, L. M., 1951b. The X organ, a neurosecretory gland controlling molting crabs. *Anat. Rec.*, **111**: 559.

Passano, L. M., 1952a. Phase contrast observations on living neurosecretory cells of *Sesarma*. *Anat. Rec.*, **112**: 460–461.

Passano, L. M., 1952b. The X organ-sinus gland complex of brachyuran crustaceans, a neurosecretory molt controlling gland. Thesis for the Ph.D. degree, Yale University.

Perkins, E. B., 1928. Color changes in crustaceans, especially in *Palaemonetes*. *J. Exp. Zool.*, **50**: 71–105.

Punt, A., 1950. The respiration of insects. *Physiol. Comp. Oecol.*, **2**: 59–74.

Saloum, R. D., 1951. The effect of the sinus gland on general metabolism as indicated by the rate of oxygen consumption in *Astacus*. Thesis for the A.B. degree, Reed College.

Scharrer, Berta, 1952a. Neurosecretion. XI. The effects of nerve section on the intercerebralis-cardiacum-allatum system of the insect *Leucophaea maderae*. *Biol. Bull.*, **102**: 261–272.

Scharrer, Berta, 1952b. Über neuroendokrine Vorgänge bei Insekten. *Pflügers Arch.*, **255**: 154–163.

Scharrer, Berta, and E. Scharrer, 1944. Neurosecretion. VI. A comparison between the intercerebralis-cardiacum-allatum system of the insects and the hypothalamo-hypophyseal system of the vertebrates. *Biol. Bull.*, **87**: 242–251.

Scharrer, E., 1951. Neurosecretion. X. A relationship between the paraphysis and the paraventricular nucleus in the garter snake (*Thamnophis* sp.). *Biol. Bull.*, **101**: 106–113.

Scholander, P. F., 1949. Volumetric respirometer for aquatic animals. *Rev. Sci. Instr.*, **20**: 885–887.

Scholander, P. F., C. L. Claff, J. R. Andrews and D. F. Wallach, 1952. Microvolumetric respirometry. Methods for measuring O_2 consumption and CO_2 production by cells and enzymic reactions. *J. Gen. Physiol.*, **35**: 375–395.

Scudamore, H. H., 1942. Influence of the sinus glands upon certain metabolic changes associated with molting in the crayfish, *Cambarus immunis*. *Anat. Rec.*, **84**: 515.

Scudamore, H. H., 1947. The influence of sinus glands upon molting and associated changes in the crayfish. *Physiol. Zool.*, **20**: 187–208.

Seidell, A., 1940. Solubilities of inorganic and metal organic compounds. Vol. I. 3rd ed., Van Nostrand Co., New York, N. Y.

Smith, R. I., 1940. Studies on the effects of eyestalk removal upon young crayfish (*Cambarus clarkii* Girard). *Biol. Bull.*, **79**: 145–152.

Travis, Dorothy F., 1951a. Calcium metabolism in the decapod Crustacea. Thesis for the Ph.D. degree, Radcliffe College.

Travis, Dorothy F., 1951b. The control of the sinus glands over certain aspects of calcium metabolism in *Panulirus argus* Latreille. *Anat. Rec.*, **111**: 503.

Welsh, J. H., 1930a. The mechanics of migration of the distal pigment cells in the eyes of *Palaemonetes*. *J. Exp. Zool.*, **56**: 459–494.

Welsh, J. H., 1930b. Diurnal rhythm of the distal pigment cells in the eyes of certain crustaceans. *Proc. Nat. Acad. Sci.*, **16**: 386–395.

Welsh, J. H., 1939. The action of eye-stalk extracts on retinal pigment migration in the crayfish, *Cambarus bartoni*. *Biol. Bull.*, **77**: 119–125.

Welsh, J. H., 1941. The sinus glands and 24-hour cycles of retinal pigment migration in the crayfish. *J. Exp. Zool.*, **86**: 35–49.

Welsh, J. H., 1951. New evidence concerning the source and action of the eyestalk hormone. *Anat. Rec.*, **111**: 442.

Williams, C. M., 1946. Continuous anesthesia for insects. *Science*, **103**: 57.

Zeleny, C., 1905. Compensatory regulation. *J. Exp. Zool.*, **2**: 1–102.